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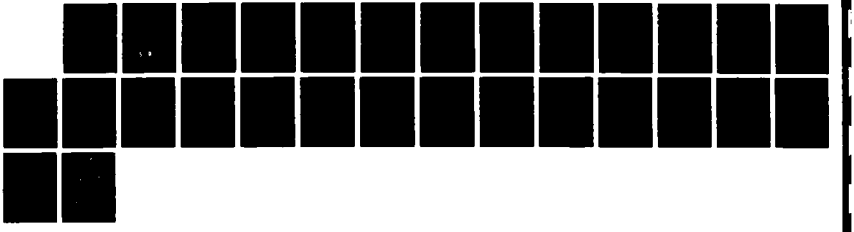

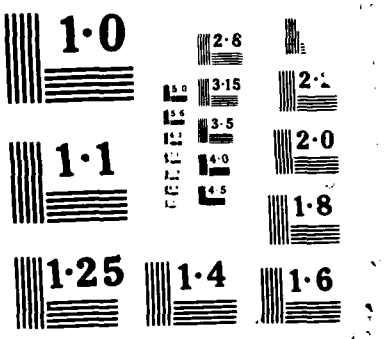
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Corticosteroid/Antibiotic Treatment of Septic Shock: An Evaluation of Mechanisms

ANNUAL/FINAL REPORT

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SUMMARY

We have succeeded in developing the first effective therapy to prevent death from septic shock induced by a 100% lethal dose of live E. coli organisms administered intravenously to dogs and nonhuman primates. The therapy consists of intermittent infusions of the corticosteroid, methylprednisolone sodium succinate, and the aminoglycoside antibiotic, gentamicin sulfate. Application of the therapy soon after initiation of E. coli administration has increased survival (> 7 days) from 0% to 100% in both dogs and baboons. The purpose of this study was to delineate the exact mechanisms of protection of our corticosteroid/antibiotic therapy, including how it is involved with the cardiovascular, metabolic, endocrinologic and host-defense systems of the septic animal. We have particularly emphasized evaluation of therapy interaction with adrenal cortex, lung, liver, and leukocytes. We have evaluated the roles of granulocytes in tissue injury in live organism-induced shock and assayed the role of corticosteroid in prevention of such injury. We have also assessed the significance of β-endorphin in the pathogenesis of shock and effectiveness of therapy. To achieve these goals using dogs, we have developed intact catheterized, adrenalectomized, and isolated working left ventricle preparations. Results underscore the following: methylprednisolone sodium succinate/gentamicin sulfate therapy does not prevent the myocardial dysfunction in E. coli-induced shock; however, corticosteroid performs a decisive role in sepsis and therapy; metabolic derangements are not the cause of death in the adrenalectomized animal challenged with LD_{100} E. coli, but early cardiovascular derangements may take precedence thus giving support to the possibility that adverse cardiovascular events precede metabolic derangements in acute severe sepsis in intact animals and humans; a decrease in phosphoenolpyruvate-carboxykinase activity contributes to the depression of hepatic and renal gluconeogenesis in lethal septic shock which ultimately leads to a metabolic death; the method, mode and timing of administration of antibiotic may prove extremely important in determining its effectiveness against the lethal effects of live organism-induced shock; short term (1-2 day) administration of large doses of corticosteroid prior to challenge with lethal E. coli causes no detrimental effects but on the contrary, may provide protection against the lethal effects of shock; however, long term daily administration (8 days) is harmful in animals subsequently challenged with E. coli primarily because the production of endogenous cortisol is severely depressed by the influence of exogenously added corticosteroid; employment of corticosteroid/antibiotic therapy in adrenalectomized dogs indicates that the therapy stimulates the cardiovascular and respiratory systems, improves peripheral perfusion, prevents loss of intravascular fluid and stimulates hepatic gluconeogenesis; the decreased assimilation of cortisol and the probability of its decreased production may explain why pharmacologic doses of exogenous corticosteroid are required for

effective treatment of E. coli-induced shock; surviving animals with intact adrenals, administered corticosteroid/antibiotic therapy, invariably demonstrate marked leukocytosis with increases in both mature and immature neutrophil concentrations, and blood glucose values are sustained at control or above control levels and diminished cortisol concentrations are present during recovery suggesting less stress and therefore less requirement for cortisol; corticosteroid/antibiotic therapy was most pronounced in its prevention of renal failure and protection of all organ systems from adverse morphologic alterations; naloxone significantly improves survival in dogs administered LD_{100} E. coli on the basis of improved multiple organ function, however baboons[1] are not protected by naloxone; divergent species responses probably explain the differences during naloxone therapy, however, after two intensive baboon studies we conclude that naloxone is without demonstrable therapeutic benefit in the baboon model of septic shock.

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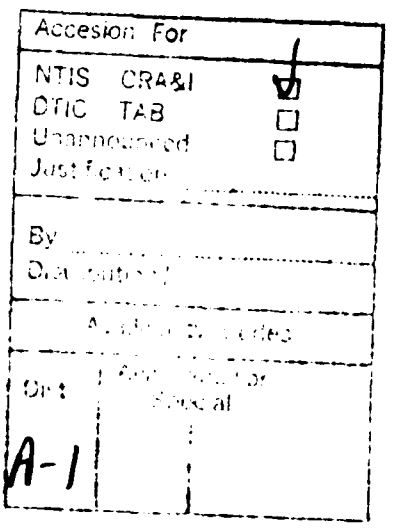
[1]baboon experiments conducted by means of separate funding



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We have succeeded in developing the first effective therapy to prevent death from septic shock induced by a 100% lethal dose of live E. coli organisms administered intravenously to dogs and nonhuman primates. The therapy consists of intermittent infusions of the corticosteroid, methylprednisolone sodium succinate, and the aminoglycoside antibiotic, gentamicin sulfate. Application of the therapy soon after initiation of E. coli

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administration has increased survival (> 7 days) from 0% to 100% in both dogs and baboons. We now propose to delineate the exact mechanisms of protection of our therapy including how it is involved with the cardiovascular, metabolic, endocrinologic and host-defense systems of the septic animal. We will particularly emphasize evaluation of therapy interaction with adrenal cortex, lung, liver, and leukocytes. We will evaluate the roles of granulocytes and complement in tissue injury in live organism-induced shock and assay the role of corticosteroid in prevention of such injury. We will also assess the significance of β-endorphins, prostaglandins and thromboxane in the pathogenesis of shock and effectiveness of therapy. Utilization of these experimental shock models provides unprecedented opportunities for developing effective understanding of the mechanisms and therapy of severe sepsis and septic shock, common sequelae of combat injuries.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of the Laboratory Animals of the Institute of Laboratory Animal Re-Sources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

"Corticosteroid/Antibiotic Treatment of Septic Shock: An Evaluation of Mechanisms

STATEMENT OF THE PROBLEM

Gram-negative septicemia occurs in approximately 1 of every 100 hospitalized patients in the United States (1, 2). The serious nature of bacteremic sepsis has been underscored by McCabe (2) who pointed out that the number of cases and deaths from bacteremia continues to increase progressively each year. Mortality rates in patients remain between 20-80% for septicemia (2, 3), and as high as 80-90% for septic shock (3-6). We have succeeded in developing the first effective therapy to prevent death (>7 days) from septic shock induced by a 100% lethal dose (LD_{100}) of live E. coli administered intravenously to dogs and baboons. The therapy consists of intermittent infusions of the corticosteroid, methylprednisolone sodium succinate (MPSS), and the aminoglycoside antibiotic, gentamicin sulfate (GS) (7-11). The problem we are addressing in this proposal is to determine the mechanisms of protection and strengths and limitations of MPSS/GS therapy.

BACKGROUND

We selected the corticosteroid, methylprednisolone sodium succinate (MPSS), to test as a therapy for experimental septic shock because we reasoned that it might correct or prevent the various defects of shock, including the maldistribution of blood flow and volume, metabolic abnormalities, coagulation and host-defense defects, and pathologic tissue changes (12-29).

We evaluated the separate and combined effects of steroid and antibiotic in dogs given LD_{100} E. coli. Infusions of MPSS and gentamicin sulfate prevented death in all dogs. Dogs that received either antibiotic or steroid alone were not protected and could not be distinguished from the group that received E. coli only (7).

Utilizing the lethal septic shock baboon model developed in our laboratory (30-31), we sequentially analyzed the effectiveness of our combination MPSS/GS regimen. Results demonstrated that the earlier the steroid/antibiotic regimen is given the more likely an animal is to survive the lethal effects of LD_{100} E. coli-induced shock. The MPSS/GS therapy prevented the hypoglycemia and corrected the hypoinsulinemia of septic shock. MPSS/GS infusion also lowered BUN blood levels, prevented anuria, lowered heart rate, and increased concentrations of circulating mature and immature neutrophils (7-10) after E. coli administration. Moreover, MPSS/GS prevented or ameliorated all significant organ damage in baboons that survived (29). These observation suggest that the responses of the adrenal gland, liver, the host-defense system, and the cardiovascular system are interdependent as they influence the outcome of septic shock. We propose to determine the mechanisms underlying these phenomena.

We are in a unique position to advance the research of septic shock and its therapy because:

1. We have developed the first treatment (MPSS/GS) that prevents the death of dogs and nonhuman primates infused with 100% lethal doses of live E. coli organisms. There are no other therapy studies using nonhuman primates challenged with live organisms that result in permanent survival.

2. Our criterion that an animal must survive more than seven days after the septic challenge to be defined a "survivor" is the most stringent survival standard in the shock literature.

3. The animal slowly infused with live organisms is a clinically relevant shock model.

4. The effectiveness of MPSS/GS treatment is not limited to a specific species since both dogs and baboons are protected.

5. Even delayed MPSS/GS therapy is effective for baboons given LD_{100} E. coli. MPSS infusion initiated 2 hours and 4 hours post onset of E. coli infusion results in survival rates of 85% and 65% respectively (9-10).

6. We now can use our well-controlled experimental animal septic models to investigate the mechanisms of protection of the MPSS/GS therapy which would be difficult to sort out in a human clinical study because of differing degrees of debilitation, various sites of infection, multiple organisms, and other factors.

APPROACH TO THE PROBLEM

A. Specific Objectives:

1. The long-term objective of this proposal is to identify the mechanisms of action of protection of our MPSS/GS therapy for LD_{100} E. coli-induced shock in the dog and baboon so that the information can be used effectively in the treatment of humans with severe sepsis and thereby prevent septic shock.

2. Short-term objectives are:

a. To determine if our MPSS/GS therapy blocks complement activation or granulocyte ability to respond to C5a in our E. coli shock models and thereby prevents or reverses granulocyte margination, aggregation, and subsequent endothelial damage.

b. To determine the role of endogenous venous exogenous corticosteroid in conjunction with antibiotic in preventing granulocyte-mediated organ damage and in preserving gluconeo-

genesis after E. coli shock.

c. To ascertain if our MPSS/GS therapy enhances or inhibits in vitro phagocytosis by mature or immature neutrophils in animals challenged with LD_{100} E. coli.

d. To determine if our MPSS/GS therapy affects the release of β-endorphins, thromboxane A_2, and protacyclin.

e. To determine whether platelet aggregation and/or release of vasoactive agents is prevented in our E. coli shock models by our MPSS/GS therapy.

f. To determine of our MPSS/GS therapy can prevent myocardial dysfunction since we have documented that the heart fails in 75% of the dogs subjected to E. coli-induced shock.

g. To determine if venus return is augmented toward control levels by MPSS/GS therapy after E. coli challenge.

h. To determine if MPSS/GS therapy in our E. coli shock models preserves renal hemodynamics and function.

i. To determine if MPSS therapy enhances microcirculatory delivery and distribution of antibiotic in our E. coli shock models.

j. To determine the strengths and limitations of our MPSS/GS therapy in E. coli shock and to delineate the times and methods of administration vital to preserve life.

B. Key Questions to Answer Are:

1. Is the maldistribution of blood volume and blood flow caused by peripheral pooling, pre- and post-capillary constriction, and decreased cardiac output in experimental septic shock prevented or reversed by our MPSS/GS therapy?

2. To what extent does endotoxin activation of complement (with subsequent margination and aggregation of WBCs, endothelial injury and disseminated intravascular coagulation) contribute to the cardiovascular dysfunction observed in septic shock and does our MPSS/GS therapy interfere with this sequence of events?

3. Does a relative adrenal insufficiency occur during septic shock and adversely affect neutrophil function (phagocytosis and killing of bacterial and glucose utilization) and liver function (gluconeogenesis)? If so, does our exogenous corticosteroid administration compensate for the adrenal insufficiency and/or prevent adrenal damage?

4. If an animal has undergone chronic steroid administration, will his response to E. coli challenge and to MPSS/GS therapy be altered?

5. Does steroid enhance the peripheral distribution of antibiotic in animals when treated with our MPSS/GS therapy? If so, is the improved distribution due to increased peripheral vascular flow?

6. Do animals survive E. coli shock when treated with our MPSS/GS therapy because the therapy prevents myocardial dysfunction?

7. Does our MPSS/GS therapy for E. coli shock prevent renal histopathology and functional impairment and enhance antibiotic excretion?

8. What are the limits of MPSS/GS therapy related to dose, time and means of administration of MPSS/GS during E. coli-induced shock?

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RESULTS, DISCUSSION AND CONCLUSIONS OF FINDINGS

Introduction

Our research findings will be discussed in terms of how they relate to achieving the objectives of the research as indicated previously. Our animal preparation is either a dog or baboon administered an LD_{100} dose of E. coli organisms to simulate human septic shock. Our treatment which is given soon after the administration of E. coli consists of intermittent infusions of the corticosteroid, methylprednisolone sodium succinate, and the aminoglycoside antibiotic, gentamicin sulfate. Application of the therapy increases survival (assessed by the 7th post-shock day) from 0% to 100% on both dogs and baboons. Experiments with baboons were supported by Veteran Administration funds and results from those experiments when appropriate to the objectives in the present contract, are also included in this report. The findings of the research are discussed in terms of the publications resulting from this project (see bibliography, page 23).

Discussion of findings

The primary objective of our research was to determine the mechanisms of protection of corticosteroid/antibiotic therapy as it is involved with cardiovascular, metabolic, endocrinologic and host-defense systems of the septic animal. We considered the role of the treatment in protecting the cardiovascular system by evaluating the myocardial function and venous return during E. coli infusion with and without corticosteroid/antibiotic therapy (2, 14). These findings are summarized as follows:

Myocardial dysfunction in endotoxin- and live E. coli-induced shock (2). Our studies have documented myocardial dysfunction within 3 to 6 hours following lethal doses of endotoxin or E. coli in dogs (2). We found that the failure was characterized by increased left ventricular and diastolic pressure, depressed peak positive and negative dP/dt, reduced myocardial efficiency and power and decreased left ventricular compliance due to increases in chamber and muscle stiffness (2). These findings led to a second study:

Evaluation of corticosteroid/antibiotic therapy for the heart in lethal septic shock (14). The purpose of this study was to determine if the treatment with steroid/antibiotic successfully preventing death in dogs given LD_{100} doses of E. coli, would lessen or abolish the degree of myocardial failure in this form of shock. Depending on the results, heart failure might be found to be a primary determinant of death during septic shock. Our initial study was conducted as follows: Dogs were anesthetized and intravenously infused for 1 hr with saline, or 1.03 ($\pm$.05) x 10^{10} E. coli/kg: Grp 1 - Saline (N=5); Grp 2 - E. coli (N=4) and Grp 3 - E. coli + MPSS + GS (N=6). MPSS and GS were

infused beginning 15 and 65 min respectively after onset of E. coli infusion. Hearts were isolated and their performance was evaluated by changing mean aortic pressure while maintaining cardiac output constant. Four to seven hours after onset of E. coli infusion severe myocardial dysfunction occurred as evidenced by increased left ventricular end-diastolic pressure (LVEDP) and decreases in negative dP/dt_{max}, power, and myocardial efficiency. LVEDP and power of hearts treated with MPSS/Gs were significantly improved from those given only E. coli and were similar to those given saline alone (no E. coli).

These data showed that MPSS/GS therapy reduced myocardial dysfunction during LD_{100} E. coli-induced shock, however, subsequent studies have not substantiated these initial observations. We considered the earlier findings highly important but were concerned about the variability of myocardial responses following steroid/antibiotic treatment. We therefore enlarged the series and found that a significant number of preparations did not show improvement of function following treatment. We are therefore concluding that corticosteroid/antibiotic treatment of animals subjected to lethal septic shock prevents pathophysiological changes by permitting benefitting the animal through means other than protecting the myocardium. These other avenues of protection have been described in recent paper (3). These benefits include prevention of amelioration of hypoglycemia, hypoinsulinemia, leukopenia, depressed microcirculatory blood flow, renal failure, and adverse morphologic changes ion lung, liver, kidney and adrenal gland (3).

We have completed a preliminary series of experiments to determine if corticosteroid/antibiotic therapy augmented venous return toward normal following E. coli infusion with the therapy given concomitantly. Preliminary results show inconclusive differences between treated and untreated animals. These studies are continuing and are considered important in as much as adequate venous return is essential for a normal cardiac output. Further, previously reported indirect evidence pointed to the possibility that corticosteroid/antibiotic administration prevents peripheral sequestration of blood and edema formation following endotoxin or E. coli challenge ion animal models.

The role of corticosteroid therapy in preventing dysfunction of the cardiovascular system, adverse changes in metabolic function and lethality in dogs challenged with lethal doses of E. coli (4). The main objective of this work was to evaluate the responses of dogs to lethal E. coli infusion when the adrenal glands of the animals are removed and their secretions are replaced by exogenously administered corticosteroid, methylprednisolone sodium succinate (MPSS). An antibiotic, gentamicin sulfate (GS), was also infused with MPSS since previous work has shown that neither intravenous infusion of MPSS nor GS alone administered to dogs with intact adrenals will prevent the

pathophysiological changes or reduce mortality following LD_{100} E. coli infusion. Results showed that adrenalectomized animals infused with MPSS and GS after the onset of lethal E. coli infusion are protected from the pathophysiological and lethal actions of E. coli. During the five hour period following the cessation of E. coli infusion, mean arterial pressure, pH, pO_2, hematocrit, lactate and glucose concentrations were maintained near control values in animals receiving steroid/antibiotic infusions. Adrenalectomized animals given E. coli plus steroid/antibiotic survived as long as adrenalectomized animals receiving no E. coli (>100 hours). It was evident that animals were spared the lethal effects of E. coli my MPSS/GS treatment and that they died later from adrenal insufficiency in a fashion very similar to those receiving no E. coli. The fact that the treated dogs recovered from the otherwise rapidly induced lethality of E. coli following only short term treatment underscores the extreme effectiveness of the therapy.

Data from these adrenalectomized animal experiments suggest that certain basic defects in the animal's response to lethal E. coli contribute to its lethal action: there is a failure to assimilate even the low concentration of plasma cortisol, and the subsequently low blood concentration of steroid triggers the development of cardiovascular and respiratory malfunctions associated with failure of peripheral perfusion, development of edema formation and depressed hepatic function. These observations may be extended to animals with intact adrenals to explain why corticosteroid/antibiotic therapy is effective, since we showed that the employment of this therapy in adrenalectomized dogs stimulates the cardiovascular and respiratory systems, improves peripheral perfusion, prevents loss of intravascular fluid and stimulates hepatic gluconeogenesis.

Comment: The possibility that plasma cortisol concentrations are adequate in the defense of the animal (or human) during septic shock is in serious question. The serum cortisol levels of animals and humans during sepsis are significantly higher that under normal conditions in most instances. Cortisol production during septic shock is undoubtedly accelerated but we believe that it is inadequate to counteract the magnitude of the stress. Plasma cortisol concentrations of dogs given lethal doses of endotoxin increase by a factor of two and those in patients who succumb to severe infections by a factor of five. However, plasma cortisol half-lives of both animals and humans are also significantly increased ($p<0.05$), particulary those of nonsurvivors, suggesting failure of cortisol assimilation. The decreased survival times of adrenalectomized dogs administered E. coli in the present study were positively correlated with both the increased plasma concentrations of cortisol and its increased half-life. This observation supports the probability that increased blood concentrations of cortisol in the shocked animal reflects a decreased rate of cortisol assimilation by tissues.

High concentrations of cortisol during septic/endotoxic shock may be partially accounted for by factors which decrease the rate of removal of cortisol from the blood including impairments of hepatocellular function, hepatic blood flow and thyroid function all of which may unite to reduce the rate of disposal of cortisol. The fact that shock produces extensive histopathology in the adrenal glands of animals and humans probably indicates that the maximal rate of adrenal secretion is depressed. The cause of adrenal gland damage during septic shock is not clearly established. Hemorrhagic necrosis with extravasation of red cells, massive hematomas, microthrombi, and platelet aggregation in varying degrees of severity have been observed in the zona glomerulose and zona fasciculate of the adrenal cortex of animals and humans. The damage may be a result of direct endotoxic or bacterial action, or may result from hypotension and/or hypoperfusion associated with substantive endogenous ACTH stimulation. The above pathophysiological changes may explain why endogenous corticosteroid secretion is inadequate to protect an animal or patient against the stress of lethal septic shock: cortisol assimilation is depressed and cortisol concentrations though significantly elevated during shock may be inadequate because of the development of extensive adrenal gland histopathology. The adrenalectomized dog challenged with E. coli has completely lost any opportunity of elevating plasma cortisol levels and moreover has the problem of decreased assimilation of the little cortisol that remains. The phenomenon of decreased assimilation of cortisol and the possibility of its depressed production, may explain why pharmacologic doses of exogenous corticosteroid are required for effective treatment of E. coli-induced shock.

Role of corticosteroid therapy as it relates to hepatic metabolism during E. coli-induced shock in adrenalectomized dogs. Introduction: During sepsis/septic shock, metabolic alterations, including glycogen depletion and diminished gluconeogenesis, occur at a time when plasma glucocorticoids, glucagon, and epinephrine levels are elevated. In addition, hypoglycemia accompanies lethal septic shock, whereas normo- to hyperglycemia is associated with survival. Steroidogenesis does not seem to be altered, but the intracellular mode of steroid action is perturbed in shocked animals. These observations emphasized the need to consider molecular events involved in the host response to acute bacterial sepsis. Our Study: We began a project designed to determine the mechanisms by which carbohydrate metabolism is perturbed during gram-negative sepsis. Our plans were to elucidate the mechanism(s) whereby combined exogenous steroid therapy and antibiotic treatment improve survival in adrenalectomized dogs administered Escherichia coli. An Adrenalectomized animal may exhibit near normal plasma glucose concentrations in basal or resting states. However, in a state of stress such as gram-negative septic shock, the ability of that animal to produce glucose is impaired. This is undoubtedly due to a failure of hepatic and renal gluconeogenesis. Control

of phosphoenolpyruvate carboxykinase (a rate-limiting gluconeogenic enzyme) synthesis is probably a major mechanism by which glucocorticoids regulate gluconeogenesis. In these ongoing studies, plasma norepinephrine concentrations increase in both control and E. coli-infused adrenalectomized dogs but the final values were not significantly different between the two groups. Results from these studies demonstrate decreased specific, high-affinity binding of ^{3}H-dexamethasone in cytosol preparations especially of liver obtained from E. coli-infused adrenalectomized dogs. Decreased binding occurred at a time after E. coli infusion when hepatic phosphoenolpyruvate carboxy-kinase (PEPCK) activity was diminished. Although steroid binding and PEPCK activity were decreased, liver glycogen content in E. coli and control animals were +18.1/5.9 and +23.3/6.7 mg/gm of liver, respectively. Insulin and glucagon values appear to be increasing in the E. coli-infused animals but the insulin/glucagon ratios and blood glucose concentrations remained relatively unchanged in both groups of animals. Conclusion: Analysis of the data to date suggest to us that although certain critical events that lead to metabolic death during lethal septic shock are manifested in the animals challenged with E. coli (e.g., decreased steroid binding and PEPCK activity), the constancy of blood glucose values reveal that in the acutely adrenalectomized animal, metabolic derangements are not the cause of death.

Evaluation of corticosteroid/antibiotic effectiveness on renal function and survival in animals subjected to lethal E. coli-induced shock (7). We completed a series of experiments to determine the effectiveness of antibiotic given as a separate treatment vd. its combination with corticosteroid (7). Adult dogs of either sex were anesthetized, divided into five groups and infused iv for one hour with Escherichia coli. Group A was given no drug. Group B was given a 45 mg/kg, 10-min iv injection of tobramycin (TOB) at 65 min. Group C was given a 3 mg/kg, 10-min TOB injection at 65 min, followed by a 8.25 mg/kg iv infusion for 285 min, and three 11.25 mg/kg intramuscular injections at 6, 12, and 18 h (total 45 mg/kg). Group D was given the same TOB regimen at B, plus a 30 mg/kg iv injection and 30 mg/kg iv infusion of methylprednisolone sodium succinate (MPSS) from 15 to 360 min. Group E was given the same TOB regimen as C, plus the same MPSS regimen as D. Treated dogs also received 11.25 mg/kg of TOB daily for days. The percent surviving more than 7 days was 0. 0. 17%, 83%, and 83%, for groups A through E, respectively. By 4 h, TOB-treated groups had significantly ($p<.05$) lower E. coli blood levels than group A. Also E. coli levels in group B were significantly ($p<0.05$) lower than those in groups C, D, or E. High trough serum TOB concentrations were associated with death and very low levels with recovery. Serum urea nitrogen and creatinine concentrations increased in all groups, but returned to normal by 7 days in survivors. Blood pressure decreased in all groups, but began to recover by 3 h in

groups D and E. Serum glucose levels of groups D and E did not decrease. Serum cortisol levels increased in all groups, but were lower than normal than 48 to 168 h in groups D and E. In summary, single-dose TOB was an effective antimicrobial agent. However, recovery from E. coli sepsis was dependent on TOB plus MPSS and was not achieved with TOB alone.

Comment: Dogs that survived E. coli sepsis had low trough TOB concentrations from 24 through 96 h, while nonsurvivors had high trough concentrations. Since all animals were given the same total amount of antibiotic, the increased trough concentrations of nonsurvivors may have been due to E. coli-induced decreases in mean systemic arterial pressure, which would decrease renal perfusion and depress renal function. Serum urea nitrogen concentrations were significantly higher in animals receiving lethal E. coli alone as compared to those given E. coli but treated with corticosteroid/antibiotic infusions. These observations emphasize the depressant effect of septic shock on renal function, which if severe enough would contribute to the development of antibiotic nephrotoxicity. Also if renal function is already depressed, the risk of antibiotic-related nephrotoxocity would increase. Possible synergism between E. coli-induced renal impairment and high antibiotic levels might further enhance the degree of nephrotoxicity. Finally, impaired microcirculatory blood flow would decrease tissue uptake of antibiotic, thereby increasing trough concentrations. We found a positive correlation between low trough concentrations of TOB, progressively decreasing serum urea nitrogen and creatinine concentrations, and increased survival rate in animals given combined corticosteroid/antibiotic therapy. Improved renal perfusion is probably a primary factor in reducing trough concentrations of antibiotic. MPSS increases renal blood flow and urine flow in endotoxin shock and decreases renal vascular resistance. Corticosteroid-induced improvements in renal and peripheral perfusion would combine to lower trough concentrations of TOB and eliminate hephrotoxicity. Low serum antibiotic concentrations in large central vessels might suggest a need for additional antibiotic. However, low concentrations may also reflect improved peripheral distribution of antibiotic and improved renal function. E. coli-induced shock studies in animals demonstrate that corticosteroid/antibiotic therapy stimulates host defense and gluconeogenesis and attenuates excessive levels of plasma lactate and serum urea nitrogen. Corticosteroid administration during endotoxemia stimulates hepatic gluconeogenesis and supports carbohydrate metabolism. All surviving animals in our study were markedly leukocytotic with increases in both mature and immature neutrophil concentrations. Blood glucose concentrations of dogs were at or above control levels. Diminished cortisol concentrations (below control values) in surviving animals may indicate less stress and therefore a diminished requirement for cortisol. It may also indicate better tissue assimilation because of improved hepatic function.

The effects of pretreatment with corticosteroid on the responses of animals subsequently receiving LD_{100} doses of E. coli (6). Dogs were given 30 milligrams per kilogram per day of methylprednisolone sodium succinate daily for one, two, or eight days then infused with 9.72 (±0.35) X 10^9 Escherichia coli per kilogram body weight. All dogs given no prior steroid, died within 25 hours. Of the dogs given one or two doses of prior steroid, 42 percent permanently survived (more than 7 days). All dogs given eight daily doses of steroid prior to Escherichia coli infusion, died within 17 hours. One or two daily doses of high-dose steroid did not detrimentally affect canine survival. Eight days of steroid administration suppressed endogenous cortisol production and when animals were treated subsequently with six hours of steroid-antibiotic therapy, survival benefits were limited.

Comment: The primary purpose of this study was to determine the effects on survival of administering high-dose steroid to dogs prior to challenging them with lethal sepsis. Dogs given no prior steroid (control group) served to substantiate the lethality (LD_{100}) of the dose of Escherichia coli; all animals died between eight and 25 hours. The survival of dogs given one or two days of 30 milligrams per kilogram per day of methylprednisolone sodium succinate prior to challenge with Escherichia coli was not altered adversely by prior steroid administration, instead, in contrast to the controls, five of 12 dogs permanently survived (more than 7 days). In contrast, dogs given injections of 30 milligrams per kilogram per day of methylprednisolone for eight days prior to challenge with Escherichia coli responded similarly to those given no steroid; all died between ten and 17 hours. We designed a fourth group of experiments to assess whether prior steroid administration had affected the dog's ability to recover from sepsis when given subsequent therapy. Six dogs were given methylprednisolone daily for eight days and then challenged with Escherichia coli and treated with the methylprednisolone and gentamicin regimen previously shown to result in 100 percent survival. Results from that group demonstrated that the resistance of the animals had been adversely affected by the eight days of prior high-dose steroid administration: only 33 percent of the dogs permanently survived. Whether the dogs were given steroid for one, two, or eight days prior to challenge with septicemia, the drug effected increases in circulating leukocyte concentrations (both in numbers of mature and immature neutrophils) and lactate concentrations and effected decreases in mean systemic arterial pressures and heart rates. It appeared that low endogenous cortisol concentration was the key factor in causing the decrease in survival. Serum cortisol values of dogs given eight daily high-dose steroid injections indicated that endogenous cortisol production was severely suppressed. After Escherichia coli infusion, those dogs had significantly lower concentrations of endogenous serum cortisol compared with dogs given only one or

two daily doses of methylprednisolone sodium succinate. This indicates that multiple doses of steroid also suppressed the dog's ability to produce cortisol when under the severe stress of sepsis. It appears to us that since eight days of high-dose steroid administration given prior to Escherichia coli infusion suppressed dogs' endogenous cortisol production particularly after Escherichia coli infusion, that six hours of methylprednisolone infusion therapy was inadequate to support the animal in overcoming the lethal effects of Escherichia coli challenge.

Evaluation of naloxone therapy for E. coli sepsis in dogs and baboons: role of β-endorphin (1, 8, 15). A final objective of our project was to assess the significance of β-endorphins, prostaglandins and thromboxane in the pathogenesis of shock and effectiveness of therapy. We have begun a study with ibuprofen to determine its effect on the pathophysiological and lethal effects of LD_{100} E. coli challenge in dogs and baboons. There was no protection of any kind in dogs, however, there appears to be a benefit in baboons. These studies are ongoing. Our research on the role of β-endorphin in septic shock has centered around its evaluation in two species: the dogs (1) and baboon (1, 8, 15) utilizing intravenous infusions of the opiate antagonist, naloxone hydrochloride. The first study in dogs and baboons is summarized as follows:

Dogs and baboons were infused intravenously (IV) with Escherichia coli and treated with the opiate antagonist, naloxone hydrochloride, and the antibiotic, gentamicin sulfate, to determine the therapeutic efficacy of naloxone. Naloxone hydrochloride (2 mg/kg) was injected IV when one fourth of the E. coli had been infused and then infused at 2 mg/kg/hr (six hours for dogs and 12 hours for baboons). Four of five naloxone-treated dogs survived permanently (greater than seven days), while all dogs that were given only E. coli died. Arterial blood pressure, blood glucose levels, pCO_2, and pO_2 were supported at higher levels and lesions of the gastrointestinal tract were prevented in naloxone-treated dogs. A steady decline in blood glucose levels after an initial hyperglycemia was observed in naloxone-treated baboons, indications of peripheral vasoconstriction were noted, and all baboons died within 42 hours.

Comment: Response of the dog to naloxone (first study, ref #1): The 0% rate of the baboons in the present study is similar to that in previous reports. In contrast, the dogs in this study were protected by naloxone. All dogs given only E. coli died within 25 hours. Four of five dogs given E. coli plus naloxone and gentamicin were permanent survivors; whereas, four of five dogs in our previous study given gentamicin only died. Dogs in the present study that were treated with naloxone were benefited in several ways compared with control animals. Mean

systemicarterial pressure was supported particulary during the later stages (five to six hours) of the study; mean heart rates were less elevated suggesting less necessity for adrenergic support; hypoglycemia was prevented; partial pressures of carbon dioxide and oxygen were higher; and the concentration of antibiotic in the peripheral venous blood at six hours was similar to the concentration in the central venous blood, which is consistent with adequate vascular distribution. The number of E. coli colony-forming units was less in the naloxone-treated group of animals than in the untreated group of animals. Gross postmortem examination of the gastrointestinal tract of the naloxone-treated animals demonstrated no significant lesions; whereas, severe hemorrhagic necrosis was observed in all dogs given E. coli only. Other investigators have observed increases in cardiac output, prevention of hypoxia in the splanchnic bed, and increases in left ventricular stroke work after the dogs were administered naloxone. The fact that the dogs in this study survived permanently, while those animals in the other studies have not may be explained, in part, by the type of shock model we utilize: The dog infused for one hour with a lethal dose of E. coli organisms undoubtedly has more time an opportunity to mobilize its host-defense systems and more time and opportunity to respond to treatment compared with the dog shock model utilizing the possibly irreversible insult of the injection of a bolus dose of lethal endotoxin. Response of the baboon to naloxone (first study, ref #1): All baboons were infused with E. coli. The baboons that were given no treatment died with 24 hours, and all baboons that were treated with naloxone and gentamicin died within 42 hours. Of the five baboons treated with gentamicin sulfate only, one baboon survived permanently (greater than seven days). The reason for the dramatic difference in survival rates between the canine and primate species in the present study is not clear. Unlike the dog, there were no significant differences in the mean systemic arterial pressure, heart rate, pCO_2, or pO_2 values of baboons given naloxone compared with the other groups. The naloxone-treated baboons responded with a rise in blood glucose concentration at two hours (significantly higher than in the other two groups), then the blood glucose concentration decreased progressively during the next ten hours. The numbers of E. coli colony-forming units were statistically lower in the two gentamicin-treated group of baboons, but, statistically, more E. coli remained in the naloxone-treated group than in the group given gentamicin only. There were several findings that, when evaluated as a whole, suggest that the baboons given naloxone in addition to gentamicin may have experienced a greater degree of peripheral vasoconstriction than the baboons given no treatment or gentamicin therapy alone. Central venous serum gentamicin concentrations in both groups of animals given antibiotic therapy were high. However, the central venous serum gentamicin concentration at 11 hours in the naloxone-treated group of animals was elevated significantly ($p<.05$), suggesting decreased

peripheral distribution and decreased renal excretion. Moreover, the central venous serum gentamicin concentration in the naloxone-treated group of animals was higher than the peripheral venous concentration at 11 hours ($p>.05$) suggesting diminished peripheral distribution of the antibiotic. The significant reduction in the number of circulating platelets at 12 hours in the naloxone-treated group of animals compared with the group of animals that were given no treatment also suggests that additional vasoconstriction, trapping increased numbers of platelets in the periphery, may have occurred. Additionally, the serum urea nitrogen concentrations increased to the same extent in all three groups of baboons, which suggests that the naloxone therapy was unable to alleviate the renal vasoconstriction associated with septic shock. Gross postmortem examination of the adrenal glands in all 12 nonsurviving baboons demonstrated moderate to massive hemorrhage. Adrenal hemorrhage has been observed consistently at autopsy in all of the E. coli-shocked nonsurviving baboons we have studied. We believe that a therapy must be able to prevent this damage to be effective. The present study provides evidence for the release of β-endorphins during E. coli-induced shock. All baboons in each of the three groups (except one in the naloxone group) demonstrated increases in plasma β-endorphin concentrations, although the overall statistical level was insignificant ($p>.05$). The mean increase in the 13 baboons was 44% ± 11% (range, -37% to 118%).

Conclusion: The sum of the findings of the experiments in this study suggest that differences in species responses may be responsible for the divergent survival data. Different species can and often do respond differently to similar stimuli. In the present study, the mechanisms of action of naloxone may have been different in the dog than in the baboon. However, a more likely hypothesis would be that the two species responded similarly to naloxone, but differently to the products of naloxone stimulation (i.e., catecholamines, if our hypothesis is correct). In contrast to the problems exacerbated in the baboon, it may be that additional catecholamine elaboration protected the dog by improving the perfusion of critical vascular beds.

The second study was carried out in baboons and is summarized as follows:

This study evaluated the therapeutic potential of the opiate antagonist, naloxone hydrochloride (NAL), used in combination with the antibiotic, gentamicin sulfate (GS), for the treatment of E. coli-induced shock in baboons. Adult baboons (N=15) were studied for 12 hrs and monitored for survival times. All baboons were intravenously infused for 2 hrs with 2.46 (±0.13) X 10^{10} E. coli/kg and treated as follows: (Grp I) E. coli + GS; (Grp II) E. coli + GS + NAL, 0.5 mg/kg bolus + 0.5 mg/kg/hr/9.5 hrs; (Grp III) E. coli + GS + NAL, 2.0 mg/kg bolus + 2.0 mg/kg/hr/6-10 hrs. NAL was administered at the end of the hyperdynamic phase when

arterial pressure had declined below baseline (>2 hours following initiation of E. coli infusion). Mean arterial pressure was elevated in baboons receiving the lower dose of NAL. However, sustained leukopenia and neutropenia were not reversed by NAL infusion. NAL potentiated the increase in plasma epinephrine produced by E. coli infusion; whereas, norepinephrine was not further increased by NAL. NAL prevented the increase in plasma β-endorphin produced by E. coli and blunted the increase in plasma cortisol. Despite these effects, NAL did not prevent multiple organ pathology and did not decrease mortality. It is concluded that naloxone is without significant therapeutic benefit in this baboon model of septic shock. Since we had previously demonstrated that NAL significantly improved survival in a dog model of E. coli sepsis, possible reasons for these different responses across species are discussed.

Comment: The present study was designed to evaluate and compare the effectiveness of two different naloxone regimens for the post-treatment of live E. coli organism-induced septicemia. Naloxone was administered to one group of baboons at the smallest bolus dosage that initially produced an increase in mean systemic pressure (0.5 mg/kg), then infused continuously at that dose until the 12th hour. Naloxone was also given to a group of baboons in a larger bolus dosage (2 mg/kg) followed by an infusion of that dose until the 6th hour. The only difference in the response of the baboons to these two protocols was the effect on blood pressure; the baboons given the smaller dose of naloxone maintained their mean arterial pressures at significantly higher values ($p<0.05$) during the 12 hour period. The differences in blood pressure between the two groups did not result in differences in survival. In fact, the percent permanent survival (20%) was the same in each group; one animal in each of the three groups survived more than 7 days. Also, survival times of nonsurvivors were not different between the three groups, averaging less than 30 hours.

The central venous serum of baboons administered naloxone showed abnormally high concentrations of gentamicin (30 ug/ml) by the 11th hour. These abnormally high values are approximately the same as those observed in our previous study in which baboons treated with naloxone after lethal E. coli infusion subsequently died. These high concentrations suggest the presence of renal dysfunction with the additional possibility of poor peripheral vascular perfusion. Results from this study indicate that the renal dysfunction and pathology caused by E. coli challenge were not prevented or attenuated by naloxone. Significant pathology was observed in 75% of the baboons. Multiple organ pathology was evident in the majority of baboons receiving naloxone treatment: adrenal hemorrhage and congestion were observed in all nonsurvivors; extensive liver, lung, and kidney pathology was seen in 75-80% of baboons and intestinal intussusceptions in relatively advanced stages were evident in most nonsurviving animals. In prior work (1), we were unable to demonstrate a therapeutic

response to naloxone in septicemic baboons despite the excellent response to naloxone in dogs subjected to septic shock. The present studies were designed to evaluate more critically the efficacy of naloxone in baboons subjected to E. coli sepsis using different doses and times of drug administration. In addition to measuring metabolic and autonomic variables, we investigated the hormonal responses to sepsis and naloxone administration. Although a smaller dose of naloxone maintained mean arterial pressure at higher values, increased survival was not attained by any dose of naloxone. Consistent with other reports, naloxone administration was associated with an increase in plasma catecholamine levels beyond the extremely high values observed in sepsis. It is possible that this effect of naloxone contributes to a further exacerbation of the sympathetic responses in baboon sepsis. Plasma β-endorphin and cortisol levels were also elevated by septicemia, and naloxone did not significantly alter these responses.

Conclusion: From these studies, we conclude that naloxone is without demonstrable therapeutic benefit in the baboon model of septic shock.

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